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S U P P O R T

Protocol for Labeling DNA by Random Oligonucleotide-primed Synthesis (with Klenow Fragment, exo-)

1. Dissolve 100ng of DNA in 10 μ l of water.
2. Add:
 - 10X reaction buffer 5 μ l,
 - 12.5 A₂₆₀units/ml random decamer primer or
7.5 A₂₆₀units/ml random hexamer primer 10 μ l,
 - deionized water to 40 μ l.
3. Incubate the mixture in a boiling water bath for 5-10 minutes and then cool immediately on ice.
4. Add:
 - 0.33mM 3dNTP mix (without labeled dNTP) 3 μ l (0.02mM - final concentration),
 - [α -³²P]-dNTP (3000Ci/mmol) 50 μ Ci,
 - Klenow fragment, exo- 5u,
 - deionized water up to 50 μ l.
5. Incubate the reaction mixture with random decamer primer at 37°C for 5 minutes and with hexamer primer for 10 minutes.
6. Add 4 μ l 0.25mM dNTP mix and incubate the reaction mixture at 37°C for 5 minutes.
7. Stop the reaction by the addition of 1 μ l 0.5M EDTA (pH 8.0).
8. Remove 1 μ l of the reaction mixture and determine the percentage of label incorporated.

Reference

1. Feinberg, A.P., Vogelstein, B., Addendum to: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Anal. Biochem.*, 137, 266-267, 1984.